Normokalemic adenosine–lidocaine cardioplegia: Importance of maintaining a polarized myocardium for optimal arrest and reanimation

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Objective: Depolarizing potassium cardioplegia does not afford optimal cardioprotection in pediatric or adult patients requiring complicated operative procedures. Polarizing adenosine–lidocaine cardioplegia has been shown to be cardioprotective without hyperkalemia. Our aim was to examine the effects of changing extracellular potassium levels in adenosine–lidocaine cardioplegia on arrest and reanimation properties.

Methods: Isolated–perfused rat hearts (n = 96) were arrested at 32°C to 33°C for 1 or 2 hours with intermittent 200 μmol/L adenosine and 500 μmol/L lidocaine in modified Krebs–Henseleit buffer with 0.1, 3.0, 5.9, 10, and 16 mmol/L potassium or with 16 or 25 mmol/L potassium in Krebs–Henseleit buffer (n = 8 for each group). Membrane potentials were estimated in the arrested ventricular myocardium (n = 42), and recovery function was measured in working mode during 60 minutes' reperfusion.

Results: Arrest was interrupted by breakout beats in the adenosine–lidocaine hypokalemic (0.1 and 3 mmol/L potassium) and non–adenosine–lidocaine hyperkalemic (16 and 25 mmol/L potassium) groups. The membrane potentials for the non–adenosine–lidocaine 16 and 25 mmol/L potassium groups were –51 and –39 mV, and those for the adenosine–lidocaine groups (0.1, 3.0, 5.9, 10, and 16 mmol/L potassium) were –183, –94, –75, –65, and –49 mV, respectively. After 1 hour of arrest, coronary vascular resistance increased linearly in adenosine–lidocaine cardioplegia with increasing potassium levels (5.9, 10, and 16 mmol/L), and the slope increased more than 2-fold after 2 hours. Nearly 40% of hearts in the adenosine–lidocaine (0.1 mmol/L potassium) and non–adenosine–lidocaine 25 mmol/L potassium groups failed to recover after 1 hour arrest. After 2 hours, hearts in the polarizing (5.9 mmol/L potassium) adenosine–lidocaine group increased coronary vascular resistance by only 30% and spontaneously recovered 107% heart rate, 92% systolic pressure, 81% aortic flow, and 113% coronary flow (all metrics returned 85% to 100% at 15 minutes) with no reperfusion arrhythmias. In contrast, hearts in the adenosine–lidocaine (3, 10, and 16 mmol/L potassium) groups were all slow to recover (15% to 40% return at 15 minutes) and experienced arrhythmias. Increasing potassium levels in adenosine–lidocaine cardioplegia from 5.9 to 16 mmol/L resulted in a 67% loss of left ventricular contractility.

Conclusions: Polarizing adenosine–lidocaine cardioplegia (5.9 mmol/L potassium) administered intermittently at 33°C provides superior arrest and reanimation profiles under normokalemic conditions when the myocardial cell membrane potential is close to its resting state. (J Thorac Cardiovasc Surg 2010;139:1576-86)

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Cardiac surgeons are currently facing unprecedented challenges and pressures from health care providers, surgeon shortages, and a sicker, older patient cohort with more diffuse multivessel disease and a higher incidence of diabetes, obesity, metabolic disorders, renal disease, failed angioplasty, and redo operations.[1] In the new era of cardiac surgery, the older patient presenting with a diseased heart without complicating comorbidities will be a surgical rarity.

High-potassium cardioplegia has been identified as a major factor associated with poor outcomes in adult bypass, valvular, or pediatric congenital corrective operations.[2,3] For more than 40 years, surgeons and scientists have sought alternatives to high-potassium cardioplegia, which arrests the heart at depolarized membrane potentials (–50 to –60 mV).[4-8] Potassium (K^+)-induced depolarization and the resulting intracellular Na^+ and Ca^{2+} loading can lead to myocardial and microvascular injury,[9] coronary vasoconstriction and spasm,[9] arrhythmias,[10] and right and left ventricular stunning[11] and potentiate the local inflammatory response with free radical production.[5,11] Apart from electrochemical arrest, hyperkalemic cardioplegia offers the patient little or no cardioprotection.

For more than 10 years, our laboratory has developed a normokalemic nondepolarizing cardioplegia to place the heart in a more natural state of suspended animation and

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Abbreviations and Acronyms

AF = aortic flow
AL = adenosine–lidocaine
CF = coronary flow
CO = cardiac output
CVR = coronary vascular resistance
HR = heart rate
K^+ = potassium
K–H = Krebs–Henseleit
SV = stroke volume

arousal akin to natural hibernation.[6] Adenosine and lidocaine, the active components of adenosine–lidocaine (AL) cardioplegia, clamp the membrane potential close to its resting voltage of –83 mV, reduce coronary vascular resistance (CVR), and improve functional recovery.[12] AL cardioplegia can arrest the heart for up to 4 hours at 29°C with small increases in CVR, 85% to 100% recovery of heart rate (HR) and systolic pressure, and 70% to 80% recovery of cardiac output (CO), whereas only 17% of hearts arrested with hyperkalemic St Thomas Hospital solution no. 2 (Plegisol; Hospira, Inc, Lake Forest, Ill) survived.[12] Further proof of concept was demonstrated by using AL all-blood cardioplegia in the canine model of cardiopulmonary bypass.[13] More recently, we have shown that AL cardioplegia can be delivered continuously or intermittently at 33°C with no significant loss in functional parameters,[7] and we are currently developing the concept for heart transplantation.[8] At nonarrest concentrations, AL intravenous infusions during regional ischemia have also been shown to reduce mortality, arrhythmias, and infarct size[14]; however, there is much controversy about whether a lidocaine bolus administered during ischemia followed by adenosine at reperfusion has similar cardioprotective effects (Canyon and Dobson, unpublished data).[15-17]

The aim of the present study was to examine the effect of varying levels of extracellular K^+ in AL cardioplegia (0.1, 3.0, 5.9, 10, and 16 mmol/L K^+) on diastolic membrane potential, CVR, incidence of arrhythmias, time to first beat, HR, systolic pressures, aortic and coronary flows (CFs), CO, and stroke volume (SV) after 1 and 2 hours of arrest at 32°C to 33°C. We report that polarized AL cardioplegia (5.9 mmol/L K^+) provides superior arrest and recovery profiles and might offer an alternative to hyperkalemic solutions in pediatric and adult cardiac surgery.

MATERIALS AND METHODS

Animals

Male Sprague–Dawley rats (390–450 g) were fed ad libitum and housed in a 14/10-hour light/dark cycle. Rats were anesthetized with an intraperitoneal injection of thiopentone sodium (100 mg/kg body weight) and handled in compliance with James Cook University Ethics approval number A1107 and with the ''Guide for care and use of laboratory animals'' from the National Institutes of Health (publication no. 85-23, Public Health Service publication 1996). Adenosine and other chemicals were obtained from Sigma Chemical Co (Castle Hill, New South Wales, Australia). Lidocaine hydrochloride was purchased as a 2% solution (ilium) from Lyppard (Queensland, Australia).

Composition of Buffers and Arrest Solutions

Krebs–Henseleit perfusion buffer. Hearts were perfused in the Langendorff and working mode with a modified Krebs–Henseleit (K–H) buffer containing 10 mmol/L glucose (117 mmol/L NaCl, 5.9 mmol/L KCl, 25 mmol/L $NaHCO_3$, 1.2 mmol/L NaH_2PO_4, 1.12 mmol/L $CaCl_2$ [free Ca^{2+} = 1.07 mmol/L], and 0.512 mmol/L $MgCl_2$ [free Mg^{2+} = 0.5 mmol/L], pH 7.4) and bubbled with 95% O_2/5% CO_2 at 37°C.[7,12]

AL arrest solutions. Adenosine (200 μmol/L) and lidocaine (500 μmol/L) were added to K–H buffer containing either 0.1, 3, 5.9, 10, or 16 mmol/L K^+ with 10 mmol/L glucose, pH 7.7.[7,12]

Hyperkalemic arrest solutions. K^+, 16 and 25 mmol/L, was added to K–H buffer containing 10 mmol/L glucose, filtered, and maintained at 37°C. The total K^+ concentration in these solutions was 16 and 25 mmol/L (as KCl). Arrest solutions were filtered with a 0.2-μm filter and not bubbled.

Experimental Protocols and Groups

Rats were randomly assigned to one of 3 experiments and 20 groups.

Cardiac membrane potential. There were 8 groups (n = 6 for each group), control (nonarrest) and immediately after arrest (at induction): AL 0.1, 3, 5.9, 10, and 16 mmol/L K^+ and 16 and 25 mmol/L K^+ alone.

One-hour arrest. There were 7 groups (n = 8 for each group): AL 0.1, 3, 5.9, 10, and 16 mmol/L K^+ and 16 and 25 mmol/L K^+ alone.

Two-hour arrest. There were 5 groups (n = 8 for each group): AL 3, 5.9, 10, and 16 mmol/L K^+ and 16 mmol/L K^+ alone. Note that we did not include AL 0.1 mmol/L K^+ or 25 mmol/L K^+ alone groups because 40% of them failed to recover after 1 hour's arrest.

Langendorff and Working Rat Heart Preparation

Hearts were placed in ice-cold K–H buffer, cleaned, and then connected through the aorta to a standard Langendorff apparatus and perfused in a retrograde fashion at 80 cm H_2O (60 mm Hg).[12] The pulmonary artery and left atrium were cannulated, and the heart was switched to working mode. The preload was 10 cm H_2O, and the afterload was 100 cm H_2O (76 mm Hg). After 10 minutes of stabilization, hearts were switched to nonworking mode to administer the arrest solution. HR, aortic pressure, CF, and aortic flow (AF) were measured before, during, and after arrest, as reported previously.[7,12] After arrest, hearts were switched to working mode, and function was monitored, as described previously.[7,12] No pacing or cardiac massage was used. The heart's temperature was measured with a Cole–Palmer temperature sensor (8402-20; Cole–Parmer, Vernon Hills, Ill) tucked under the left auricle.[7] Breakout beats were monitored visually, timed, and recorded during induction of arrest and during and between maintenance cardioplegic flushes. Heart rhythm was not assessed with an electrocardiogram, but irregularities in rhythm were noted, together with their effect on HR, developed pressures, and CO.

Arrest Induction Protocol

Fifty milliliters of cardioplegia was administered at 60 mm Hg, and the aorta was crossclamped for 18 minutes, after which a 2-minute infusion pulse of cardioplegia was administered and the clamp was reapplied. Cardioplegic volumes were measured for estimating CVR.[7] The arrest temperature of 32°C to 33°C was chosen from the studies that reported that tepid temperatures provided optimal heart recovery and brain protection during cardiac surgery.[7] Estimation of myocardial membrane potential and CVR can be found in Appendix 1.

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Statistical Analysis

All results are expressed as means ± standard errors of the mean. Data from the 1- and 2-hour protocols were analyzed separately. One-way analysis of variance was used to compare data at specific time points (eg, arrest time and time to first reperfusion beat; Tables 1 and 2 and Figures 1, *B*; 2, *A* and *B*; and 3, *A*). Two-way analysis of variance with repeated measures was used to compare functional parameters of the different cardioplegia groups across multiple time points during the arrest period and 60 minutes of reperfusion (Figures 1, *B*, and 3, *A* and *B*). Significance was assessed with Tukey and Dunnett (2-sided) post-hoc tests. The alpha level of significance for all experiments was set at $P < .05$.

RESULTS

Arrest Times and Membrane Potentials

Times to arrest for the AL cardioplegia groups were not significantly different and ranged from 7.4 ± 0.6 to 11 ± 1.3 seconds. Arrest times were significantly increased in hearts perfused with 16 mmol/L K^+ alone (22 ± 2 to 28 ± 8 seconds) and decreased in the presence of 25 mmol/L K^+ alone (13.4 ± 1.1 seconds). Nearly all hearts perfused with hypokalemic AL solutions (0.1 and 3 mmol/L K^+) failed to maintain arrest; breakout beats occurred for 9.8 ± 1.3 minutes and 7.1 ± 0.6 minutes, respectively. Similarly, hearts perfused with 16 mmol/L K^+ alone and 25 mmol/L K^+ alone had breakout beats lasting for 2.5 to 25 minutes and for 8 seconds to 11 minutes, respectively, after which arrest occurred. The membrane potential for each group is found in Figure 1, *A*, and Table 1. The prearrest value was –78 ± 1 mV at 37°C. Total tissue water content was not significantly different among the groups (86.0 ± 0.4% to 87.1 ± 0.5%, n = 6 each).

CVR During Induction and 2-Minute "Flushes"

During induction, there were no significant differences in the rate of coronary outflow in the AL groups, although a 5% increase occurred in the AL 5.9 mmol/L K^+ group. In contrast, the rates in the 16 mmol/L and 25 mmol/L K^+ alone groups were significantly decreased (24% and 35% decrease, respectively; Table 1). The significant increase in CVR for the 16 mmol/L K^+ group is also shown on the y-axis of Figure 1, *B* (''end of induction'').

Maintenance cardioplegic volumes and CVR measured during 2-minute flushes every 18 minutes over 1 and 2 hours' arrest are shown in Table 1 and Figure 1, *B*. Significantly greater volumes were delivered to the AL 5.9 mmol/L K^+ and AL 10 mmol/L K^+ groups. After 1 hour's arrest, the AL 0.1 mmol/L K^+ and 25 mmol/L K^+ alone groups had the highest CVRs. Nearly 40% (3/8) of hearts in each of these 2 groups subsequently failed to recover aortic/coronary flows after 1 hour's arrest, and therefore we did not include them in the longer arrest study. After 2 hours' arrest, the AL 5.9 mmol/L K^+ group had the lowest CVR (Figure 1, *B*). As the membrane potential deviated from near-resting voltage (ie, from AL 5.9 mmol/L

TABLE 1. Arrest parameters: 1- and 2-hour warm arrest protocols (32°C–33°C)

		1-h Arrest			2-h Arrest		
			Coronary vascular resistance (megadyne · s^{-1} · cm^{-5})			Coronary vascular resistance (megadyne · s^{-1} · cm^{-5})	
Arrest solution	Diastolic membrane potential (n = 6)	Cardioplegic flush volume (mL)*	End induction	At 58 min arrest	Cardioplegic flush volume (mL)*	End induction	At 118 min arrest
AL (0.1 mmol/L K^+)	–183 ± 1 mV	74 ± 2	0.25 ± 0	0.53 ± .02#	ND	ND	ND
AL (3.0 mmol/L K^+)	–94 ± 1 mV	87 ± 3	0.26 ± 0.01	0.40 ± 0.03	138 ± 5	0.25 ± 0.01	0.65 ± 0.06
AL (5.9 mmol/L K^+)	–75 ± 2 mV	104 ± 1†	0.24 ± 0.01	0.29 ± 0.01	203 ± 3‡‡	0.24 ± 0.01	0.31 ± 0.01|||
AL (10 mmol/L K^+)	–65 ± 1 mV	109 ± 3‡	0.24 ± 0.01	0.28 ± 0.02	155 ± 4	0.27 ± 0.01	0.47 ± 0.03
AL (16 mmol/L K^+)	–49 ± 1 mV	89 ± 3	0.26 ± 0.01	0.40 ± 0.06	157 ± 6	0.28 ± 0.01	0.57 ± 0.07
16 mmol/L K^+	–51 ± 1 mV	71 ± 2	0.35 ± 0.02||	0.46 ± 0.03**	139 ± 5	0.40 ± 0.03§§	0.57 ± 0.05
25 mmol/L K^+	–39 ± 1 mV	56 ± 2§	0.38 ± 0.02¶	0.61 ± 0.05††	ND	ND	ND

The diastolic membrane potential of the left ventricle was calculated from the Nernstian distribution of potassium ions (see the Materials and Methods section). *AL*, Adenosine–lidocaine; *ND*, not determined), AL 0.1 mmol/L potassium (K^+) or 25 mmol/L K^+ alone were not determined because approximately 40% failed to recover function after 1 hour arrest.
*Volume of cardioplegic solution administered during 2-minute flushes during the maintenance period.
†$P < .01$ compared with AL 0.1 mmol/L K^+ and 16 mmol/L K^+ groups.
‡$P < .01$ compared with AL 0.1 mmol/L K^+ and 16 mmol/L K^+ groups, $P < .05$ compared with AL 3 and 16 mmol/L K^+ groups.
§$P < .01$ compared with AL 3, 5.9, 10, and 16 mmol/L K^+ and 16 mmol/L K^+ groups, $P < .05$ compared with the AL 0.1 mmol/L K^+ group.
‖$P < .01$ compared with all groups except 25 mmol/L K^+.
¶$P < .01$ compared with all groups except 16 mmol/L K^+.
#$P < .05$ compared with AL 5.9 and 10 mmol/L K^+ and 25 mmol/L K^+ groups.
**$P < .01$ compared with AL 5.9 and 10 mmol/L K^+ groups.
††$P < .05$ compared with all other groups.
‡‡$P < .05$ compared with AL 3 mmol/L K^+ and 16 mmol/L K^+ groups.
§§$P < .01$ compared with all other groups.
‖‖‖$P < .01$ compared with AL 3 and 16 mmol/L K^+ and 16 mmol/L K^+ groups.

TABLE 2. Functional parameters of isolated rat hearts before arrest and at 60 minutes' reperfusion (working mode) after 1 or 2 hours' arrest at 32°C to 33°C

	Heart rate		Systolic pressure		Diastolic pressure		Aortic flow		Coronary flow		Cardiac output		Stroke volume	
Treatment groups (n = 8 each group)	**beats/min**	**% PA**	**mm Hg**	**% PA**	**mm Hg**	**% PA**	**mL/min**	**% PA**	**mL/min**	**% PA**	**mL/min**	**% PA**	**mL/beat**	**% PA**
AL (0.1 mmol/L K^+)														
Before arrest	338 ± 6	61	133 ± 2	54	75 ± 3	66	56 ± 2	28	22 ± 1	41	78 ± 2	32	0.23 ± 0.1	32
1-h Arrest	206 ± 61		71 ± 21		51 ± 15		16 ± 6¶		9 ± 3†		25 ± 9		0.07 ± 0.02	
AL (3 mmol/L K^+)														
Before arrest	336 ± 15	99	129 ± 4	92	71 ± 3	105	57 ± 2	81	20 ± 1	90	77 ± 2	83	0.23 ± 0.1	84
1-h Arrest	329 ± 7		118 ± 3		74 ± 2		46 ± 2		18 ± 1		64 ± 3		0.19 ± 0.01	
Before arrest	304 ± 10	45	131 ± 3	23	61 ± 1	32	60 ± 2	11	20 ± 1	17	79 ± 3	13	0.26 ± 0.1	13
2-h arrest	133 ± 43*		32 ± 18*		20 ± 10*		6 ± 5‖		4 ± 2‖		10 ± 7‖		0.04 ± 0.02‖;	
AL (5.9 mmol/L K^+)														
Before arrest	301 ± 19	110	131 ± 4	94	73 ± 2	100	56 ± 2	91	19 ± 1	101	75 ± 3	93	0.25 ± 0.1	86
1-h Arrest	327 ± 16		124 ± 4		73 ± 2		51 ± 3		19 ± 1		70 ± 3		0.22 ± 0.01	
Before arrest	291 ± 11	107	130 ± 1	92	61 ± 1	100	59 ± 2	81	18 ± 1	113	77 ± 2	89	0.26 ± 0.1	84
2-h Arrest	309 ± 9		120 ± 2		61 ± 1		48 ± 2#		21 ± 1#		69 ± 2#		0.22 ± 0.01#	
AL (10 mmol/L K^+)														
Before arrest	320 ± 10	102	132 ± 2	94	71 ± 2	110	60 ± 1	79	20 ± 1	97	79 ± 1	84	0.25 ± 0.1	83
1-h arrest	325 ± 9		124 ± 2		78 ± 3		47 ± 1		19 ± 1		66 ± 2		0.21 ± 0.01	
Before arrest	338 ± 15	95	124 ± 1	92	60 ± 1	107	56 ± 1	71	19 ± 1	81	75 ± 2	74	0.23 ± 0.1	80
2-h arrest	317 ± 16		114 ± 2		64 ± 1		40 ± 2		16 ± 1		56 ± 1		0.18 ± 0.01	
AL (16 mmol/L K^+)														
Before arrest	309 ± 15	103	131 ± 2	93	71 ± 2	101	56 ± 1	81	19 ± 1	94	75 ± 1	84	0.25 ± 0.1	82
1-h arrest	318 ± 15		122 ± 3		72 ± 3		45 ± 2		18 ± 2		18 ± 2		0.20 ± 0.01	
Before arrest	309 ± 14	64	139 ± 2	53	61 ± 1	65	57 ± 2	29	19 ± 1	33	76 ± 2	36	0.25 ± 0.1	39
2-h Arrest	208 ± 33		74 ± 23		39 ± 11		20 ± 8		8 ± 3		28 ± 11		0.10 ± 0.04	
16 mmol/L K^+ alone														
Before arrest	329 ± 6		133 ± 2		73 ± 3		59 ± 1		19 ± 1		78 ± 2		0.24 ± 0.1	
1-h arrest	306 ± 25	94	117 ± 3	88	78 ± 3	107	40 ± 5	69	15 ± 1	81	55 ± 5	72	0.18 ± 0.01	76
Before arrest	304 ± 9		134 ± 3		61 ± 1		56 ± 1		18 ± 1		75 ± 2		0.25 ± 0.1	
2-h arrest	248 ± 47	76	74 ± 21	56	41 ± 12	65	25 ± 8	40	10 ± 3	49	35 ± 10	46	0.11 ± 0.03	43
25 mmol/L K^+ alone														
Before arrest	306 ± 12		136 ± 3		66 ± 3		59 ± 1		20 ± 1		79 ± 1		0.26 ± 0.1	
1-h arrest	189 ± 56†	63	78 ± 23‡	57	44 ± 13§	66	26 ± 8†	43	11 ± 3†	54	37 ± 11**	46	0.12 ± 0.04†	46

%*PA*, Percentage of prearrest value; *AL*, adenosine–lidocaine.
*$P < .01$ compared with AL 5.9 and 10 mmol/L potassium (K+) groups.
†$P < .05$ compared with AL 3, 5.9, 10, and 16 mmol/L K^+ groups.
‡$P < .05$ compared with AL 5.9 and 10 mmol/L K^+ groups.
§$P < .05$ compared with AL 3 and 10 mmol/L K^+ and 16 mmol/L K^+ alone groups.
‖$P < .01$ compared with the AL 10 mmol/L K^+ group.
¶$P < .01$ compared with all groups except the 25 mmol/L K^+ alone group.
#$P < .01$ compared with the AL 3 and 16 mmol/L K^+ and 16 mmol/L K^+ alone groups.
**$P < .01$ compared with the AL 3, 5.9, 10, and 16 mmol/L K^+ groups.

K^+), the CVR increased, and this effect was magnified as arrest time increased (Figure 1, *C*).

Time to First Spontaneous Beat and Aortic Flow

After 1 hour's arrest, times to first beat increased from 0.9 minutes (AL 0.1 mmol/L K^+) to 5.2 minutes (25 mmol/L K^+ alone; Figure 2, *A*). The effect of doubling crossclamp time led to longer times to first beat, with the exception of the AL 5.9 mmol/L K^+ and AL 3.0 mmol/L K^+ groups (Figure 2, *A*). Time to first AF increased from 3.1 minutes (16 mmol/L K^+ alone) to 13.9 minutes (AL 16 mmol/L K^+; Figure 2, *B*). Doubling arrest time resulted in up to 2.4 longer times to generate AF (Figure 2, *B*). Nineteen hearts failed to achieve AF in our study: AL 0.1 mmol/L K^+ (3 hearts after 1 hour's arrest), AL 3 mmol/L K^+ (6 hearts after 2 hours' arrest), AL 16 mmol/L K^+ (4 hearts after 2 hours' arrest), 16 mmol/L K^+ alone (3 hearts after 2 hours' arrest), and 25 mmol/L K^+ alone (3 hearts after 1 hour's arrest). AL 5.9 mmol/L K^+ and AL 10 mmol/L K^+ were the only 2 groups in which

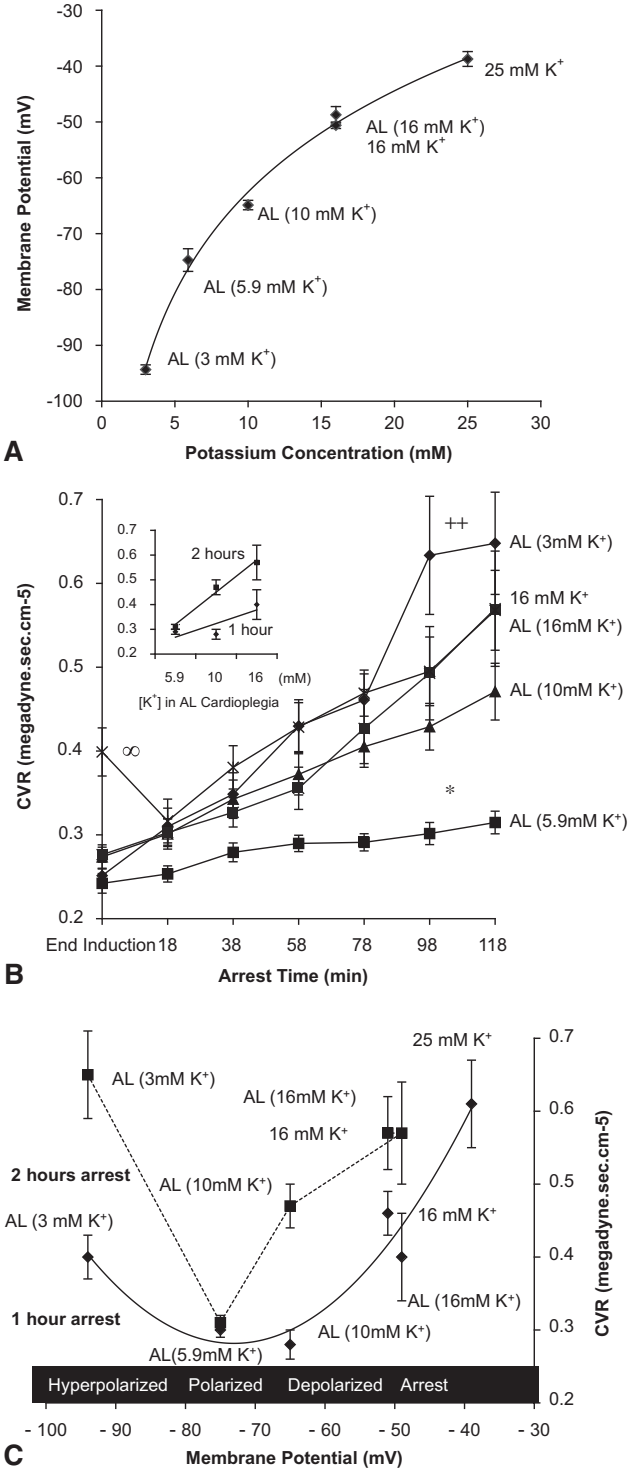

FIGURE 1. A, The effect of increasing potassium (K^+) concentrations on the left ventricular diastolic membrane potential in the isolated Langendorff rat heart arrested with adenosine–lidocaine *(AL)* cardioplegia (3.0, 5.9, 10, and 16 mmol/L K^+) or 16 mmol/L K^+ alone and 25 mmol/L K^+ alone in Krebs–Henseleit buffer. Membrane potentials (φ) were calculated from the Nerstian distributions of K^+ (see the Materials and Methods section for details). The relationship between diastolic membrane potential and extracellular K^+ is as follows: φ (mV) = 26.23 ln [K^+] − 123.44 (R^2 = 0.99), where [K^+] is the extracellular K^+ concentration in millimoles per liter. B, The effect of arrest time on coronary vascular resistance *(CVR)* during intermittent cardioplegic flushes (2minutes every 18minutes) over a 2-hour arrest period (1-hour arrest groups are not shown). ∞, 16 mmol/L K^+ alone, $P < .01$ (1-way analysis of variance) compared with AL 3.0, 5.9, 10, and 16 mmol/L K^+. ++, AL 3.0 mmol/L K^+, $P < .05$ (1-way analysis of variance) compared with AL 10 mmol/L K^+. *, AL 5.9 mmol/L K^+, $P < .05$ (by repeated measures) compared with AL 3 and 16 mmol/L K^+ and 16 mmol/L K^+ alone. C, The effect of variation in the membrane potential on coronary vascular resistance *(CVR)* for groups arrested for 1 and 2hours at 32°C to 33°C. The AL 0.1 mmol/L K^+ group (φ = –183 mV) and the 25 mmol/L K^+ alone group (φ = –39 mV) were not included in the 2-hour arrest groups because nearly 40% failed to return aortic flow after 1hour arrest. *Insert*, Comparison of CVR after 1 or 2 hours' arrest with AL containing 5.9, 10, or 16 mmol/L K^+. CVR after 1 hour's arrest was as follows: CVR = 0.0116 [K^+ mmol/L] +0.2004 (R^2 = 0.78). That after 2hours was as follows: CVR = 0.0251 [K^+] + 0.1834, R^2 = 0.94.

all hearts recovered AF after 1 and 2 hours' arrest at 33°C. The effect of membrane potential and increasing K^+ concentrations in AL cardioplegia on time to first beat and AF are shown in Figures 2, *C* and *D*.

Early Reperfusion Ventricular Arrhythmias

During reanimation and before AF was achieved, abnormal rhythms were observed in hearts from the AL 0.1 mmol/L K^+ (2 hearts), 16 mmol/L K^+ alone (1 heart), and 25 mmol/L K^+ alone (2 hearts) groups arrested for 1 hour, and after 2 hours in the AL 10 mmol/L K^+ (3 hearts), AL 16 mmol/L K^+ (4 hearts), and 16 mmol/L K^+ alone (2 hearts) groups.

Functional Recovery

Recovery of HR, systolic and diastolic pressures, aortic and coronary flows, CO, and SV for the 1- and 2-hour arrest groups are shown in Table 2 and Figure 3. During prearrest, there were no significant differences between the groups. After 1 and 2 hours' arrest, the AL 5.9 mmol/L K^+ group recovered 107% to 110% of its prearrest HR at 60 minutes' reperfusion (94% to 98% of recovery occurred at 15 minutes), which was significantly higher than that seen in the AL 3, 10, and 16 mmol/L K^+ groups after 2 hours' arrest. After 1 hour's arrest, the AL 10, 16, and 3 mmol/L K^+ and 16 mmol/L K^+ alone groups recovered 84%, 70%, 79%, and 99% of their prearrest HR at 15 minutes' and 102%, 103%, 99%, and 94% at 60 minutes' reperfusion, respectively. After 2 hours' arrest, the AL 10 mmol/L K^+ group recovered 95% at 60 minutes; however, only 36% recovery occurred at 15 minutes. The 16 mmol/L K^+ alone, AL 16 mmol/L K^+, and AL 3 mmol/L K^+ groups recovered 79%, 28%, and 39% of their respective pre-arrest HRs at 15 minutes' and 76%, 64%, and 45% at 60 minutes' reperfusion (Table 2). The relationship between HR and increasing extracellular K^+ concentrations in AL cardioplegia at 15 minutes' reperfusion after 1 hour's arrest was as follows: HR

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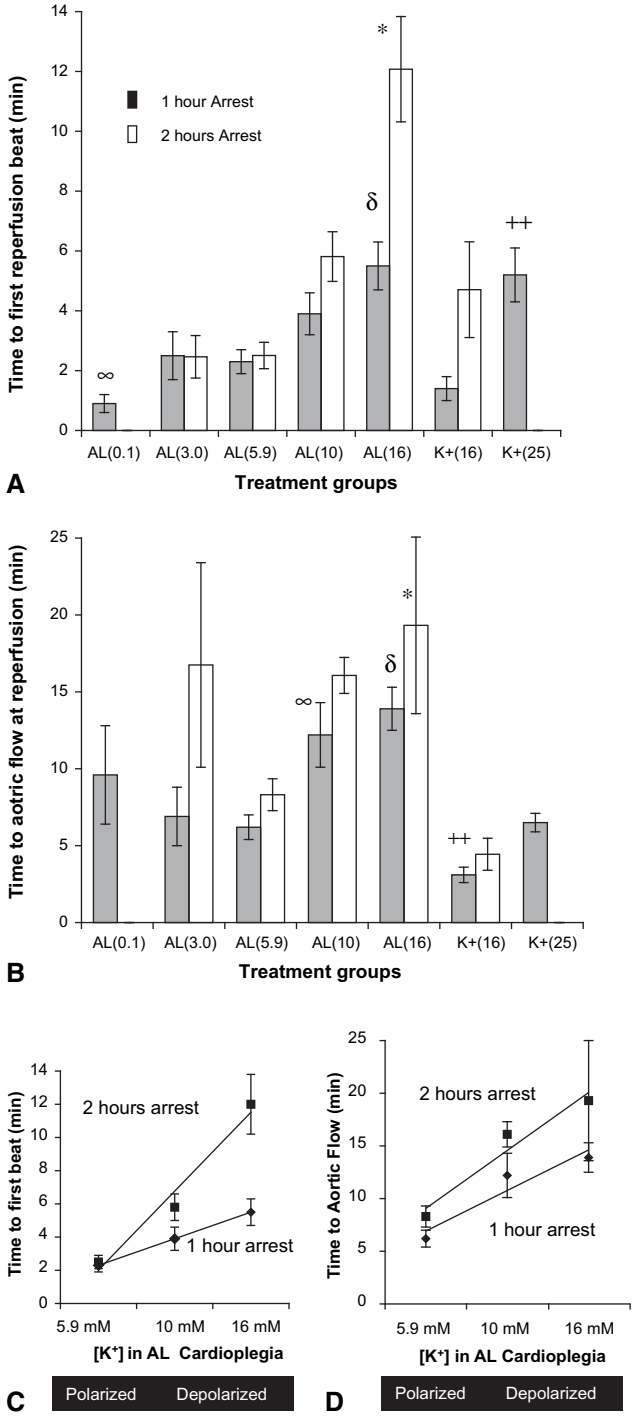

FIGURE 2. A, Time to first ventricular beat at reanimation in working mode after 1 hour's *(shaded)* or 2 hours' *(not shaded)* arrest. Adenosine–lidocaine (AL) 0.1mmol/L potassium (K^+) and 25 mmol/L K^+ alone were not included in the 2-hour arrest study because approximately 40% failed to recover function after 1 hour's arrest (same for panel B). ∞, AL 0.1 mmol/L K^+, $P < .05$ compared with AL 10 and 16 mmol/L K^+ δ, AL 16 mmol/L K^+, $P < .05$ compared with AL 3 and 5.9 mmol/L K^+ and 16 mmol/L K^+ alone. *, AL 16 mmol/L K^+, $P < .01$ compared with all other groups. ++, 25 mmol/L K^+ alone, $P < .05$ compared with AL 0.1 and 5.9 mmol/L K^+ and 16 mmol/L K^+ alone. B, Time to aortic flow at reperfusion after 1 hour's *(shaded)* or 2 hours' *(not shaded)* arrest. ∞, AL 10 mmol/L K^+, $P < .01$ compared with 16 mmol/L K^+ alone. δ, AL 16 mmol/L K^+, $P < .05$ compared with AL 3 and 5.9 mmol/L K^+. *, AL 16 mmol/L K^+, $P < .05$ compared with AL 5.9 mmol/L K^+ and 16 mmol/L K^+ alone. ++, 16 mmol/L K^+ alone, $P < .01$ compared with AL 10 and 16 mmol/L K^+. C, Relationship between the time to first beat at reanimation after 1 or 2 hours' arrest and the concentration of K^+ in AL cardioplegic solution (5.9, 10, and 16 mmol/L K^+). Time to first beat after 1hour was as follows: Time to first beat (min) = 0.3131 $[K^+] + 0.5703$, ($R^2 = 0.99$). That after 2hours was as follows: Time to first beat (min) = 0.9474 $[K^+] - 3.3076$ ($R^2 = 0.99$). D, Relationship between the time to aortic flow after 1 or 2 hours' arrest and the concentration of K^+ in AL cardioplegic solution (5.9, 10, and 16 mmol/L K^+). Time to aortic flow after 1hour was as follows: Time to aortic flow (min) = 0.727 $[K^+] + 3.3035$ ($R^2 = 0.84$). That after 2hours was as follows: Time to aortic flow (min) = 1.057 $[K^+] + 3.290$ ($R^2 = 0.88$).

(percentage recovery) = –3.8406 [K^+ mmol/L] + 128.84 (R^2 = 0.92). That after 2 hours' arrest was as follows: HR (percentage recovery) = –6.1517 [K^+ mmol/L] + 118.08 (R^2 = 0.75).

After 1 and 2 hours' arrest, hearts in the AL 5.9 mmol/L K^+ group demonstrated a rapid return of systolic and diastolic pressures, with nearly 100% return at 15 minutes and 92% to 100% recoveries at 60 minutes (Table 2). After 1 hour's arrest, the AL 10 mmol/L K^+ and 16 mmol/L K^+ alone groups recovered 90% to 100% developed pressures at 15 minutes; however, in contrast to the AL 5.9 mmol/L K^+ group, the AL 10 mmol/L K^+ group was slow to recover after 2 hours' arrest, with only 50% developed pressures at 15 minutes. After 2 hours, the AL 16 mmol/L K^+ and 16 mmol/L K^+ alone groups recovered 43% to 66% developed pressures at 15 minutes, and the AL 3 mmol/L K^+ group recovered only 17% to 26% at 15 minutes; developed pressures for these groups changed little over the next 45 minutes (Table 2).

After 1 and 2 hours' arrest, the AL 5.9 mmol/L K^+ group recovered 81% to 91% of its prearrest AF at 60 minutes (Table 2), and nearly 100% return occurred at 15 minutes. After 2 hours' arrest, this recovery for the AL 5.9 mmol/L K^+ group was significantly higher than for all other groups (Table 2). After 1 hour's arrest, the AL 10, 16, and 3 mmol/L K^+ and 16 mmol/L K^+ alone groups recovered 57%, 44%, 71%, and 75%, respectively, of prearrest AF at 15 minutes and 79%, 81%, 81%, and 69% at 60 minutes (Table 2). Hearts in the 25 mmol/L K^+ alone group recovered significantly less, with 43% AF at 60 minutes (49% return at 15 minutes). Hearts in the AL 0.1 mmol/L K^+ group recovered 28% (15% at 15 minutes). After 2 hours' arrest, the AL 10 mmol/L K^+ group recovered 71% at 60 minutes but showed only 26% return at 15 minutes. The 16 mmol/L K^+ alone, AL 16 mmol/L K^+, and AL 3 mmol/L K^+ groups recovered 33%, 15%, and 4%, respectively, of their prearrest AF at 15 minutes (Table 2). The relationship between AF and increasing extracellular K^+ concentrations in AL cardioplegia

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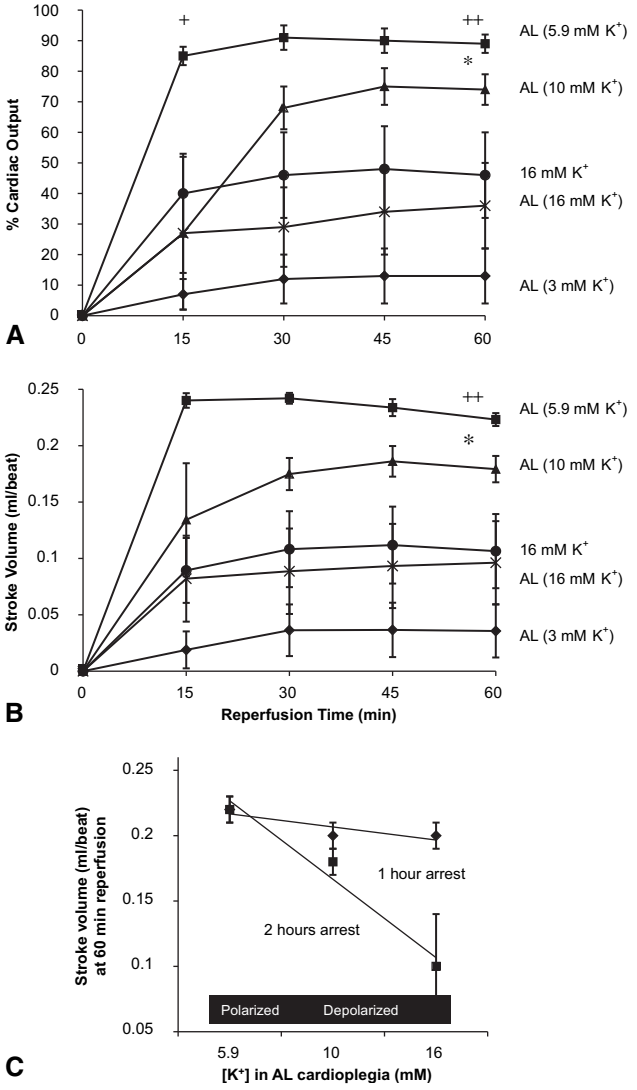

FIGURE 3. A, Recovery of cardiac output (percentage of prearrest values) during 60 minutes' reperfusion after 2 hours' arrest at 32°C to 33°C.+, Adenosine–lidocaine *(AL)* 5.9 mmol/L K^+, $P < .01$ (1-way analysis of variance) compared with AL 3, 10, and 16 mmol/L potassium (K^+) and 16 mmol/L K^+ alone. ++, AL 5.9 mmol/L K^+, $P < .01$ (1-way analysis of variance) compared with AL 3 mmol/L K^+, $P < .05$ (repeated measures) compared with AL 3 and 16 mmol/L K^+ and 16 mmol/L K^+ alone. *, AL 10 mmol/L K^+, $P < .01$ (repeated measures) compared with AL 3 mmol/L K^+. B, Recovery of stroke volume (in milliliters per beat) during 60 minutes' reperfusion after 2 hours' arrest at 32°C to 33°C.++, AL 5.9 mmol/L K^+, $P < .01$ (repeated measures) compared with AL 3 and 16 mmol/L K^+ and 16 mmol/L K^+ alone. *, AL 10 mmol/L K^+, $P < .01$ (repeated measures) compared with AL 3 mmol/L K^+. C, Relationship between stroke volume and increasing concentrations of K^+ in AL cardioplegic solution (5.9, 10, and 16 mmol/L K^+) at 60 minutes' reperfusion for 1- and 2-hour arrest groups. Stroke volume after 1hour was as follows: Stroke volume = 0.0018 [K^+ mmol/L]+0.2262 ($R^2 = 0.65$). That after 2hours was as follows: Stroke volume = 0.012 [K^+]+0.294 ($R^2 = 0.99$).

at 15 minutes' reperfusion after 1 hour's arrest was as follows: AF (percentage recovery) = –4.4704 [K^+ mmol/L] + 111.53 ($R^2 = 0.88$). That after 2 hours' arrest was as follows: AF (percentage recovery) = –6.1885 [K^+ mmol/L] + 106.47 ($R^2 = 0.79$).

Recovery of rate and percentage of CF showed profiles similar to those of AF (Table 2). After 1 and 2 hours' arrest, the AL 5.9 mmol/L K^+ group recovered 101% to 113% of its prearrest CF at 60 minutes, and 95% of this recovery was achieved at 15 minutes' reperfusion. After 1 hour's arrest, the AL 10, 16, and 3 mmol/L K^+ and 16 mmol/L K^+ alone groups recovered 72%, 65%, 72%, and 78%, respectively, of prearrest CF at 15 minutes and 97%, 94%, 90%, and 81% at 60 minutes' reperfusion. Hearts arrested with 25 mmol/L K^+ alone recovered only 54% (49% at 15 minutes), and those arrested with AL 0.1 mmol/L K^+ recovered 41% (45% at 15 minutes) of their prearrest CF. After 2 hours' arrest, the AL 10 mmol/L K^+ group recovered 81% at 60 minutes, and only 40% of recovery occurred at 15 minutes. The 16 mmol/L K^+ alone, AL 16 mmol/L K^+, and AL 3.0 mmol/L K^+ groups recovered 44%, 29%, and 15% of their prearrest CF at 15 minutes' and 49%, 33%, and 17% at 60 minutes' reperfusion, respectively (Table 2). The relationship between CF and increasing extracellular K^+ concentrations in AL cardioplegia at 15 minutes' reperfusion after 1 hour's arrest was as follows: CF (percentage recovery) = –2.8375 [K^+ mmol/L] + 107.51 ($R^2 = 0.84$). That after 2 hours' arrest was as follows: CF (percentage recovery) = –6.1885 [K^+ mmol/L] + 120.47 ($R^2 = 0.79$).

The recovery of CO and SV are shown in Table 2 and Figure 3. After 1 and 2 hours' arrest, the AL 5.9 mmol/L K^+ group recovered 89% to 93% of its prearrest CO at 60 minutes (85% to 93% of this recovery at 15 minutes; Figure 3, *A*). After 1 hour's arrest, the AL 10, 16, 3 mmol/L K^+ and 16 mmol/L K^+ alone groups recovered 61%, 49%, 71%, and 76% of prearrest CO at 15 minutes' and 84%, 84%, 83% and 72% at 60 minutes' reperfusion, respectively. Hearts arrested with 25 mmol/L K^+ alone recovered only 46% (49% at 15 minutes), and those arrested with AL 0.1 mmol/L K^+ recovered 32% prearrest CO. The relationship between CO and increasing extracellular K^+ concentrations in AL cardioplegia at 15 minutes' reperfusion after 1 hour's arrest was as follows: CO (percentage recovery) = –4.1829 [K^+ mmol/L] + 112.15 ($R^2 = 0.87$). After 2 hours' arrest, the AL 10 mmol/L K^+ group recovered 74% of prearrest value and 27% at 15 minutes' reperfusion, which was significantly less than that seen in the AL 5.9 mmol/L K^+ group. The 16 mmol/L K^+ alone, AL 16 mmol/L K^+, and AL 3.0 mmol/L K^+ groups recovered 40%, 27%, and 7% of prearrest CO after 15 minutes' and 46%, 36%, and 13% at 60 minutes' reperfusion, respectively (Figure 3, *A*, and Table 2). Similar recovery profiles were found with SV (Figure 3, *B*). After 2 hours' arrest, the AL 5.9 mmol/L K^+ group recovered 0.22 ± 0.1 mL/beat (84% of prearrest SV) and 0.24 ± 0.1 mL/beat (90% recovery was achieved at 15 minutes). This recovery was significantly higher than that seen in the AL 3 mmol/L K^+, AL 16 mmol/L K^+, and 16 mmol/L K^+ alone groups.

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DISCUSSION

Surgeons have known for decades that depolarizing cardioplegia does not afford optimal cardioprotection.[2,3] A number of years ago, we introduced an alternative arrest, protection, and preservation concept using nondepolarizing normokalemic AL.[7,8,12] The aim of the present study was to examine the effects of varying the concentration of extracellular K^+ in AL cardioplegia on arrest and reanimation properties in the isolated working rat heart at warm temperatures. We show that warm AL cardioplegia is most efficient under normokalemic conditions when the myocardial cell membrane potential is close to its resting state. Hearts arrested with lower K^+ concentrations (hyperpolarizing cardioplegia) or higher K^+ concentrations (depolarizing cardioplegia) had significantly higher CVRs during arrest, experienced reanimation arrhythmias, and were slow to recover, with significant losses in CO, SV, and contractility. Nearly 40% of hearts in the AL 0.1 mmol/L K^+ and 25 mmol/L K^+ alone groups failed to return HR, developed pressures, or CO after 1 hour's arrest.

Effect of Changing Extracellular K^+ Concentrations on Cardiac Membrane Potential

The theoretic relationship between resting membrane potential and transmembrane K^+ concentration gradient was first proposed in 1902 by German physiologist Julius Bernstein,[18] after Ostwald and Nernst. We report a Nernstian relationship between changing K^+ concentrations and membrane potential (φ) in the arrested heart: φ (mV) = 26.23 ln [K^+ mmol/L] − 123.44 ($R^2 = 0.99$; Figure 1, *A*). This finding is in good agreement with our previous work on the rat heart[12,19] and with the large number of independent microelectrode measurements made on in vivo, isolated heart and myocyte preparations.[4,20,21] For example, Snabaitis and colleagues[4] measured –50 mV in the isolated rat heart perfused with 16 mmol/L K^+ alone in K–H buffer (n = 8), and Kleber[20] measured –82 ± 2 mV in the isolated guinea pig heart perfused with 4.5 mmol/L K^+ (n = 6). From Figure 1, *A*, our values for Snabaitis and colleagues'[4] and Kleber's[20] measurements are –50.7 and –84 mV, respectively, at identical K^+ concentrations. Similarly, at lower K^+ concentrations (3.0 mmol/L), Wan and coworkers[21] measured –93 mV using microelectrodes in ventricular myocytes of guinea pigs, and our value from Figure 1, *A*, is –94 mV and in good agreement given the limitations of both methods. In the present study we did not estimate the membrane potential throughout the arrest period but only at induction. Snabaitis and colleagues[4] showed that membrane potential remained relatively constant during polarized and nonpolarized arrest time in the rat heart receiving tetrodotoxin and 16 mmol/L K solution, respectively. It would be of interest to conduct future studies with both microelectrode measurements and Nernstian K^+ distributions during arrest induction, maintenance, and recovery. Because the Nernst equation strictly describes an equilibrium state, our data lend support to the concept that the resting membrane potential in the heart is not a diffusion potential but arises from a measure of electrical work.[6,22] Our data indicate that the net myocardial transmembrane flux of K^+ over the range of 0.1 to 25 mmol/L K^+ is zero, which means during diastolic arrest, the net inward current (eg, sum of rectifier IK1)[23] is equal to the net reversal potential for K^+ ions where $\triangle G' [K^+]_{o/i} = 0$.[19,22] In conclusion, despite decreasing the transmembrane gradient by increasing extracellular K^+ concentrations from 5.9 to 25 mmol/L (increasing depolarization), as is widely used to induce arrest in cardiac surgery, our study indicates that the heart membrane potential follows a Nernstian relationship.

Effect of Changing K^+ and Membrane Potential on CVR During Arrest

Hypokalemia. We showed that hypokalemic AL arrest (0.1 and 3.0 mmol/L K^+) led to significantly higher CVR values (up to 2.6-fold after 2 hours' arrest) compared with AL normokalemia (Figure 1, *B*). In 1974, Brace and associates[24] reported that an intracoronary hypokalemic (2.1 mmol/L K^+) infusion into the canine heart in vivo under constant pressure led to reduced blood flow and increased oxygen consumption. They concluded that the increase in CVR was either the result of active vasoconstriction (direct vascular smooth muscle contraction), passive vasoconstriction (extravascular compression), or both.

Normokalemia. The CVR for AL cardioplegia with 5.9 mmol/L K^+ increased by only 30% over 2 hours' warm arrest (0.24 to 0.31 ± 0.01 megadyne · s^{-1} · cm^{-5}, n = 16). Brace and associates[24] reported that a small K^+ window exists between approximately 5 and 10 mmol/L at which reduced CVR and dilation occurs; however, we did not investigate this window in the present study.

Hyperkalemia. For many decades, hyperkalemia has been linked to increased CVR, endothelial dysfunction, and vasospasm.[9,25,26] We confirmed our earlier results in the isolated rat heart showing that hyperkalemia increases CVR during warm arrest[8,12] and that CVR was linearly related to extracellular K^+ in AL cardioplegia (from 5.9 to 16 mmol/L; Figure 1, *B*, insert). It is noteworthy that the rate of change in CVR with increasing K^+ (slope) was more than 2 times higher with doubling of arrest time (Table 1 and Figure 1, *B*, insert). In terms of membrane potential, hyperpolarization (–183 and –94 mV) or depolarization (–65, –49, and –39 mV) increases CVR to a significantly greater extent than does normokalemic AL membrane polarization (Figure 1, *C*). We conclude that diastolic arrest voltages outside the normal physiologic limits have an untoward effect on CVR and might reduce cardioplegia distribution and compromise protection during surgical arrest and reanimation.

Time to First Beat and AF and Reperfusion Arrhythmias

Time to first spontaneous ventricular beat also increased linearly with extracellular K^+ concentrations in AL cardioplegia (Figure 2, *A* and *C*). After 1 hour, time to first beat increased from 2.3 to 5.5 minutes, and after 2 hours, it increased from 2.5 to 12 minutes (Figure 2, *A* and *C*). Interestingly, the difference in slopes in Figure 2, *C*, shows that time to first spontaneous ventricular beat (in minutes) per millimole per liter of K^+ is 3 times longer after 2 hours' arrest and importantly that time to first spontaneous ventricular beat for AL (5.9 mmol/L K^+) was unchanged (Figure 2, *C*). Similar relations were found for time to first AF (Figure 2, *B* and *D*). Increasing K^+ concentrations also increased the incidence of abnormal heart rhythms during reanimation. Hearts arrested with AL 5.9 mmol/L K^+ cardioplegia for 1 and 2 hours' arrest were the only groups in which all hearts showed continual and consistent spontaneous beating with no arrhythmias in early reperfusion (Data not shown).

Recovery of Pump Function

Hearts arrested with normokalemic AL (5.9 mmol/L K^+) cardioplegia were also superior in functional recovery of HR, developed pressures, AF, CF, CO, and SV compared with those hearts receiving hypokalemic or hyperkalemic AL cardioplegia or 16 and 25 mmol/L K^+ alone cardioplegia (Table 2 and Figure 3).

We found that after 1 and 2 hours' warm arrest, the AL 5.9 mmol/L K^+ cardioplegia groups recovered rapidly and predictably 90% to 110% of HR, developed pressures, AF, CF, and CO (Table 2 and Figure 3, *A*). After 2 hours, hearts receiving depolarizing AL 10 mmol/L K^+ cardioplegia recovered function slower, with significantly lower CO at 15 minutes' reperfusion (Table 2 and Figure 3, *A*). Increasing the K^+ concentration to 16 mmol/L in AL cardioplegia led to further functional loss in recovery of HR, developed pressures, AF, CF, and CO (Table 2 and Figure 3, *A*). Interestingly, nearly all metrics changed in a linear fashion with increasing K^+ concentrations (from 5.9 to 16 mmol/L) at 15 and 60 minutes' reperfusion (Table 2 and Figure 3). Furthermore, doubling the arrest time exacerbated the damaging effects of K^+ (see the Results section). The lower functional recoveries for the AL 10 and 16 mmol/L K^+ or 16 and 25 mmol/L K^+ alone groups might be due to K^+-induced myocardial (and vascular) stunning through H^+ ion imbalances, oxidative stress, calcium overload, and mitochondrial oxidative dysfunction.[11]

The effect of increasing K^+ concentrations in AL cardioplegia on loss of functional recovery was highlighted by a decrease of up to 67% in SV (Figure 3, *B*). SV (in milliliters per beat) was calculated by dividing CO by HR, and because the preload (10 cm H_2O) and afterload (100 cm H_2O) are preset in the working heart preparation, a decrease in SV might indicate a decrease in left ventricular contractility. The relationship between SV and increasing K^+ concentrations in the AL cardioplegia after 1 hour's arrest at 15 minutes' reperfusion was as follows: SV (mL/beat) = –0.0085 [K^+ mmol/L] + 0.2771 (R^2 = 0.84). That after 2 hours was as follows: SV (mL/beat) = –0.0152 [K^+ mmol/L] + 0.3103 (R^2 = 0.88). After 2 hours, SV decreased by up to 67% at 15 minutes' and 60% at 60 minutes' reperfusion, and the rate of decrease was more than 10-fold compared with that seen in the 1-hour arrest group at 60 minutes' reperfusion (Figure 3, *B* and *C*). We conclude that increasing K^+ concentrations from 5.9 to 10 and 16 mmol/L in AL cardioplegia results in a significant loss of CO, SV, and left ventricular contractility, and the decrease is more pronounced the longer the arrest time. Pressure–volume loops or an intraventricular balloon would be required to quantify the effects of AL with varying K^+ concentrations on changes in contractility (eg, degree of sarcomere stretch) during recovery. In cell voltage terms the more the cardiomyocyte, heart conduction cells, and cells of the coronary vasculature appear to deviate from their natural resting membrane potentials during cardioplegic arrest, the greater the loss of cardiac function during arrest, reanimation, and reperfusion.

Possible Clinical Significance: Polarized Versus Depolarized Cardioplegia

Although the underlying mechanisms of AL likely involve an interaction of adenosine receptors, Na^+ fast channels, protein kinase C survival pathways, and adenosine triphosphate–sensitive K^+ channels (mitochondrial and sarcolemmal),[12] the key to polarized arrest and related polarizing cardioprotection strategies is that there are fewer voltage-dependent membrane channels, pores, and exchangers (Na^+/H^+ and Na^+/Ca^{2+}) open compared with depolarized states.[5,12] Depolarizing K^+ cardioplegia has been a double-edged sword in cardiac surgery for more than 4 decades, particularly after Tyers and colleagues[27] demonstrated unequivocally that K^+, and not citrate, was responsible for the clinical failure of Melrose's method. Currently, there are at least 5 clinical concerns with depolarizing K^+ cardioplegia. These include (1) generating unnatural voltages and ionic gradients in the heart leading to cell Na^+ and Ca^{2+} loading,[11] which are exacerbated with longer crossclamp times[7,12]; (2) coronary vasoconstriction leading to higher resistances, suboptimal delivery pressure–flow relations, and nonuniform distribution of cardioplegia[28,29]; (3) damage to the vascular endothelium and smooth muscle interactions, which predispose the coronary vessels to spasm[9,30]; (4) the occurrence of postoperative atrial and ventricular arrhythmias, including conduction disturbances[10]; and (5) ventricular dysfunction, such as postcardioplegia stunning.[11]

Because polarizing normokalemic AL cardioplegia maintains cell voltages close to resting values, this strategy might offer surgeons an alternative and address the growing

concern about the lack of cardioprotection in high-risk patients undergoing cardiac surgery or those with extended crossclamp times. Although the present study was conducted on healthy hearts, future studies need to be conducted on older hearts with comorbidities and neonatal hearts. A few clinical data are available on crystalloid and all-blood AL cardioplegia in pediatric and adult cardiac surgery. Jin and associates[31] reported that a ''one shot'' of AL 10 mmol/L K^+ crystalloid cardioplegia was more protective than AL 20 mmol/L K^+ or non-AL 20 mmol/L K^+ cardioplegia alone in 134 pediatric patients undergoing correction of ventricular septal defect. With decreasing K^+ concentrations, these authors showed that as the membrane potential shifted toward more polarized levels (ie, from –46 to –67 mV), there was (1) a reduced use of inotropes, (2) a significantly lower release of troponin I after surgical intervention, and (3) 1 day less of hospitalization.[31] Our data in the present study help to explain some of the advantages of physiologic levels of K^+ in AL cardioplegia.

Another clinical case study using all-blood AL (or Adenocaine; Hibernation Therapeutics, Wulguru, Australia) cardioplegia was reported for a 4× redo high-risk patient with infective endocarditis requiring mitral and aortic value replacements with aortic root enlargement. During cardiopulmonary bypass (9.8 hours, with a total arrest time of 7 hours), the patient's heart was kept quiescent with 72 L of all-blood Adenocaine microplegia (with 2 mmol/L K^+) for nearly 5 hours. The patient required no balloon pump and a low level of inotropic support, was extubated in 12 hours, and was transferred from the intensive care unit after 48 hours with a subsequent uneventful recovery.[32] Another possible advantage for normokalemic AL cardioplegia would be in patients with renal disease or those receiving dialysis who cannot tolerate fluctuations in plasma K^+ concentrations.

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Appendix 1

Estimation of Myocardial Membrane Potential

Left ventricular tissue of prearrest and arrested hearts at induction was freeze-clamped at liquid N_2 temperatures, ground in a mortar, and stored at –80°C until used. Samples of tissue (50–100 mg) were digested with nitric acid and analyzed for total tissue K^+ concentrations (mg/kg) with a Varian Liberty Series II Inductively Coupled Plasma Atomic Emission Spectrometer (Melbourne, Australia).

Membrane potential (V_M or φ in mV) was calculated from the Nernstian distribution of K^+ ion between the extracellular and intracellular phases (equation 1).

$$V_M = E_{K^+} = \frac{RT}{Z_{K^+}F} \times \ln \frac{[K^+]_{OUT}}{[K^+]_{IN}} \quad (1)$$

where R is the universal gas constant (8.31 J mol^{-1} K^{-1}), F is Faraday's constant (96.49 KJ mol^{-1} V^{-1}), T is absolute temperature (305.15 K), z is the valence of K^+ ion (+1), and $[K^+]_{IN}$ and $[K^+]_{OUT}$ are the intracellular and extracellular concentrations of the K^+ ion in moles per liter, respectively.[12] The $[K^+]_{IN}$ was calculated from the following equation: $[K^+]_{TOTAL} = x\ [K^+]_{IN} + y\ [K^+]_{OUT}$, where x is the intracellular space and y is the extracellular space, respectively. In the perfused working rat heart, the distribution of total tissue water is 41% intracellular and 59% extracellular.[19] It was assumed that $[K^+]_{OUT}$ was equal to the K^+ concentrations in the various cardioplegic solutions (0.1, 3.0, 5.9, 10, 16, or 25 mmol/L K^+). Total tissue water was measured and calculated from the difference between wet and dry weight, divided by wet weight, and multiplied by 100.[19]

Coronary Vascular Resistance

CVR (megadyne · s^{-1} · cm^{-5}) was calculated by dividing delivery pressure (mm Hg) by flow (volume/s) from equation 2 as follows:

$$CVR = \frac{1333 \times mm\ \text{Hg}}{(mL/s)} \times 10^{-6} \quad (2)$$

where 1 mm Hg = 1333 dynes · cm^{-2}, and 10^{-6} is a conversion factor from dynes to megadynes.[12]